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Variation of protein MWD parameters and their associations with free asparagine concentration and quality characteristics in hard red spring wheat☆

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ABSTRACT

The lower free asparagine concentration (FAC) in wheat is better since it is a precursor to form carcinogenic acrylamide during baking. This research was performed to determine the variations of protein molecular weight distribution (MWD) parameters and the associations of protein MWD parameters with quality characteristics and FAC using eleven hard red spring (HRS) wheat genotypes grown at three locations in North Dakota. Among MWD parameters, the polymeric proteins of SDS unextractable fraction were found to be useful in screening HRS wheat genotypes for low FAC and improvement of bread-making quality characteristics. The ANOVA indicated that growing locations and genotypes significantly ($P < 0.01$) influenced variation of SDS unextractable polymeric protein parameters while effect of genotype by location interaction was non-significant ($P > 0.05$). The quantity of SDS unextractable polymeric proteins had significant and positive genotypic correlations (r_g) with quality characteristics including mixograph pattern ($r_g = 0.87$, $P < 0.01$) and bread loaf volume ($r_g = 0.86$, $P < 0.01$). The ratio of SDS unextractable polymeric proteins to total protein had a negative correlation with FAC ($r_g = -0.92$, $P < 0.01$). These results supported the conclusion that the genotypic variations were primarily associated with the significant ($P < 0.05$) correlations of SDS unextractable polymeric protein parameters with mixograph pattern, bread loaf volume, and FAC.

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1. Introduction

The protein molecular weight distribution (MWD) parameters have been found to be useful for screening wheat genotypes for bread-making quality in hard red spring (HRS) wheat (*Triticum aestivum* L.) (Gupta et al., 1993; Ohm et al., 2009; Simsek et al., 2016; Tsilo et al., 2010). The sodium dodecyl sulfate (SDS) buffer extractable proteins (EXP) and unextractable proteins (UNP) were analyzed for MWD using a size exclusion-HPLC (SE-HPLC). The polymeric proteins in EXP and UNP, which are mostly composed of glutenins linked by disulfide bonds (Larroque et al., 1997), have been identified distinctly as the primary components affecting the dough/gluten viscoelastic property in HRS wheat (Ohm et al., 2009; Simsek et al., 2016; Tsilo et al., 2010). The SDS extractable and unextractable polymeric proteins have been especially thought to have different associations with dough mixing characteristics for HRS wheat genotypes. The quantitative variation of polymeric proteins in UNP has been shown to associate positively with dough mixing stability, while those in EXP had negative associations based on the simple linear correlation analysis for HRS wheat genotypes (Ohm et al., 2009; Tsilo et al., 2010).

Dietary intake of acrylamide is known to risk human health due to acrylamide's carcinogenicity (EPA, 2009; Friedman, 2003). Baked products have been identified as a prime source of dietary intake of acrylamide in the U.S. and other countries (FDA, 2009; Friedman, 2003; Grob, 2007). Free asparagine is a main component that forms acrylamide during baking (FDA, 2009; Mottram et al., 2002; Stadler et al., 2002). One of the most efficient ways to reduce acrylamide formation in wheat-based baked products is to breed

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wheat genotypes that have low levels of free asparagine concentration (FAC) (Claus et al., 2006; Curtis et al., 2009; Friedman and Levin, 2008; Taeymans et al., 2004). A significant variation in FAC was reported for HRS wheat genotypes grown in ND that suggests the practicality of breeding of low level FAC wheat cultivars (Ohm et al., 2017b). However, FAC analysis is not ideal for screening experimental lines in wheat breeding due to the time-consuming and complex analytical procedure. The protein MWD parameters were identified to have significant associations with FAC in HRS wheat samples (Klindworth et al., 2014; Liu et al., 2011; Ohm et al., 2016; Simsek et al., 2014). However, the results were based on research performed using special sample sets including flour milling streams (Liu et al., 2011), wheat damaged by artificial sprouting (Simsek et al., 2014), durum translocation lines (Klindworth et al., 2014), and germinated wheat samples (Ohm et al., 2016). Further research is necessary to substantiate that protein MWD parameters may be useful indirect indexes for screening genotypes for low FAC in HRS wheat breeding.

The interaction of genotype by environment (GE), as well as the main effects of genotype and environment, is a major source of variation for quality traits in wheat. When a trait shows large variation for genotypes without a significant GE interaction it might be easier to screen genotypes for the trait. Very little data has been recently reported on the variation of protein MWD parameters, especially for the effect of GE interaction, in HRS wheat grown in North Dakota. Genotypic correlation estimates the degrees of shared genetic association between two traits that may be heritable and, therefore, is useful to identify a certain trait that can be utilized as an indirect selection index to predict variance of the other trait in breeding program. To the best of our knowledge, there has been no research with respect to the effect of genotypes on associations of protein MWD parameters with quality characteristics and FAC in HRS wheat.

The main goal of this research was to evaluate the applicability of the protein MWD parameters to screening wheat genotypes for low FAC and breadmaking quality. The specific objectives of this research were to investigate the effects of genotype, location, and interaction of genotype by location on variation of protein MWD parameters; and to estimate genotypic correlations of the protein MWD parameters with quality characteristics and FAC for HRS wheat genotypes grown in North Dakota.

2. Materials and methods

2.1. Materials

Wheat samples were composed of two cultivars (Glenn and Faller) and nine experimental lines (V01-V09) of HRS wheat grown at three locations (Carrington, Casselton, and Langdon in ND). These samples were selected from an advanced yield trial in 2010, as they might represent variations for quality characteristics of HRS wheat in ND. The field arrangement was a randomized complete block design with 4 replicates. Whole-wheat flour samples were milled from individual wheat samples attained from three replicates, using a Udy mill with a 0.8 mm screen. Quality analyses were performed for the wheat samples that were composited by combining four replication samples.

2.2. Size-exclusion HPLC

Proteins were extracted in one mL of SDS buffer (0.5% SDS in 0.1M sodium phosphate buffer, pH 6.9) for EXP and UNP (Gupta et al., 1993). The EXP fraction was extracted from whole wheat flour sample (10 mg, 12% mb) using a pulsing vortex mixer (Fisher Scientific) and then the UNP fraction was obtained after solubilizing the proteins in the residue with a sonicator (Sonic Dismembrator 100, Fisher Scientific) (Gupta et al., 1993; Ohm et al., 2009). The MWD of proteins was analyzed by an Agilent 1100 Series chromatograph (Agilent Technologies) using the size exclusion micro bore column (2.0 × 150 mm, Shodex) with a guard cartridge (Bio-Sep SEC S4000, Phenomenex) (Ohm et al., 2017a). The SE-HPLC settings were as follows: injection volume, 2 μL; eluting solution, 50% acetonitrile in aqueous 0.1% trifluroacetic acid solution; flow rate, 0.12 mL/min; and detection, absorbance at 214 nm (Photodiode array detector, Agilent Technologies).

In-house MATLAB (R2015b, The MathWorks) program was employed to process SE-HPLC data (Ohm et al., 2009, 2017a). Absorbance area (AA) and area percentage (A%) values were calculated at a 1.5 s interval using absorbance data interpolated at a retention time interval of 0.3 s. The chromatograms were separated into three main fractions (F1, 1.5–2.5 min, F2, 2.5–3.3 min and F3, 3.3–4.9 min). The main components were shown to be glutenin polymers for F1, gliadins for F2, and other monomeric proteins such as albumins and globulins for F3 by Larroque et al. (1997). The A% values of individual fractions were converted into protein percent based on wheat weight (W%) using wheat protein content (Park et al., 2006). The simple linear correlation coefficient (r) was estimated between FAC and A% values for individual retention time intervals (1.5 s) and presented as continuous spectrum over retention time (Ohm et al., 2009, 2017a).

2.3. Free asparagine

Free asparagine was extracted from whole-wheat flour (0.2 g, 14%, mb) using 10 mL of HCl solution (0.01 M) (Muttucumaru et al., 2006; Liu et al., 2011). Amino acid analysis was performed according to the EZ-Faast procedure (Phenomenex, Torrance, CA) following the procedure described by Liu et al. (2011) using a gas chromatography system (HP 5890 Series II, Hewlett Packard, Palo Alto, CA) and a mass selective detector (HP 5971, Hewlett Packard, Palo Alto, CA).

2.4. Quality analysis

Wheat samples were cleaned on a Carter Day XT5 seed cleaner (Simon-Carter, Minneapolis, MN) and analyzed for quality characteristics. Test weight was measured by a Dicky-John GAC 2100 instrument (Dickey-John, Auburn, IL). Whole-wheat protein concentration was determined by near-infrared spectroscopy (Infratec 1226 Grain Analyzer, Foss Tecator, Höganäs, Sweden). Flour extraction was done using a Quadrumat Sr. mill. Wheat samples that were tempered to 16% moisture for 18 h were milled at the feed rate of 150 g/min. The mixograph was performed with a 10 g bowl (AACCI Approved Method 54–40.02). Mixograph water absorption was determined by the following formula listed in the AACCI Approved Method: % absorption = (1.5 × % protein) + 43.6. The mixograph pattern was evaluated using a scale of 1–10 where 1 is very weak and 10 is very strong. Experimental bread-making was performed to evaluate flour baking quality. Pup loaves (25 g) were baked according to AACCI Approved Method 10–09.01 with minor modifications (Baasandorj et al., 2015).

2.5. Statistical analysis

Statistical analysis was performed using SAS (V. 9.2, SAS Institute, Cary, NC). For protein MWD parameters, the MIXED procedure in SAS was used to test significance for the effects of genotype, growing location, and their interaction with option of DDFM = Satterth; considering genotype and location as fixed effects. The replication was considered nested in locations. The FAC

data converted to natural logarithm were statistically analyzed by ANOVA since the Bartelett test indicated non-homogenous error variance (Curtis et al., 2009). The genotypic mean values were estimated by the LSMEAN procedure. Difference of mean values was evaluated by the least significant difference. For quality traits, ANOVA was performed considering interaction of genotype by growing locations as an error term, since quality analyses were performed using the composite samples of four replications. Phenotypic and genotypic correlation coefficients (r_p and r_g, respectively) were estimated using the SAS code written by Holland (2006). The mean of standard error values that were calculated between variables of which variance was significant at $P < 0.10$ for the genotypes were used to estimate probability levels of r_p and r_g, respectively (Ohm et al., 2017b).

3. Results and discussion

3.1. Quality characteristics

The mean, standard error (SE), minimum, and maximum values of quality parameters are given in Table 1. Variation of test weights were significant ($P < 0.05$) for growing locations and genotypes, showing larger range for genotypes than locations. Mean wheat protein content was 14.8% with SE values of 1.4 for locations and 0.6 for genotypes. The higher SE value for locations reveals that protein quantitative variation was larger for locations than genotypes. Mixograph pattern, which is indicative of gluten viscoelasticity, showed significant difference only for genotypes. The significant variation of mixograph parameters were also reported for HRS wheat genotypes by Tsilo et al. (2010).

For bread-making parameters, locations showed difference for bread loaf volume and crumb color while genotypes varied significantly only for water absorption. Variations of bread-making parameters have usually been assessed to be significant for locations and genotypes in HRS wheat (Tsilo et al., 2010). When evaluated based on dough handling and bread loaf volume, the bread-making quality for all the HRS wheat genotypes was quite excellent in this research. Non-significant ($P > 0.05$) variations of those bread-making quality characteristics might result from the narrow ranges for HRS genotypes in this research.

The FAC values appeared to vary significantly among locations and genotypes when using the FAC values converted to natural logarithms (Table 1). The FAC showed ranges of 503–781 (μg/g) for locations and 346.9–886.2 (μg/g) for genotypes. Among HRS wheat genotypes, Glenn was identified to have the least FAC among genotypes. The significant variation of FAC was also reported among genotypes and locations in previous research using a larger sample set of HRS wheat (Ohm et al., 2017b). The genotypes also appeared to vary significantly for FAC in previous research (Klindworth et al., 2014; Simsek et al., 2014). Gao et al. (2016) identified four types of asparagine synthetase gene that are likely to be responsible for asparagine variation in wheat.

3.2. Variation of protein MWD parameters

Means of protein MWD parameters are given in Table 2. The mean percent wheat protein (W%) values of the SE-HPLC protein fractions which represent the quantity of individual protein fractions in the wheat were significantly lower for Casselton than other locations. There was significant ($P < 0.01$) variation of W% values for F1, F2, and F3 in EXP (EF1, EF2, and EF3, respectively) and F1 in UNP (UF1). The same ANOVA test showed non-significant ($P > 0.05$) variation for F2 and F3 in UNP (UF2 and UF3, respectively) among growing locations (Table 3). Genotypes were significantly ($P < 0.01$) different for EF1 and UF1 of which the main component was identified to be polymeric proteins (Larroque et al., 1997) (Table 3). Notably, one cultivar, Glenn, showed higher UF1 than the other genotypes in the test (Table 2). Growing locations appeared to have higher influence on quantitative variation of two polymeric protein parameters (EF1 and UF1) for ANOVA showed larger mean square values for location than genotypes (Table 3). The EF2 of which main component is known to be gliadins (Larroque et al., 1997) also varied significantly ($P < 0.001$) for locations and genotypes. Especially, EF2 showed larger variance for growing locations than other fractions, indicating that gliadins mainly accounted for quantitative variation of proteins due to the effect of locations (Table 3). However, the variance of EF2 in genotypes was not as conspicuous as for locations as shown by the lower mean square for genotypes than locations.

The A% values could be equivalent to the proportion of individual protein factions in the total protein (Park et al., 2006). The mean values of A% values of protein fractions did not show any significant difference between locations (Table 2), indicating that protein composition varied by a small degree for growing locations despite the strong effect on quantitative variation. For A% values, EP1, EP3 and UP1 were varied significantly for genotypes (Table 2). Specifically, the cultivar, Glenn, had higher UP1 than other genotypes while showing a lower level of EP1. Genotypes had larger variance for EP1 and UP1 than growing locations (Table 3).

The interaction effect of genotype by location was not significant ($P > 0.05$) for all the protein MWD parameters except for EF3 (Table 3). This result indicates that individual genotypes were

Table 1
Mean, standard error (SE), minimum (Min), and maximum (Max) values of quality traits and free asparagine concentration (FAC) in hard red spring wheat genotypes grown at three locations in North Dakota.

		Location (n = 3)				Genotype (n = 11)			
Quality Characteristics	Mean	SE	Min	Max	LSD[a]	SE	Min	Max	LSD[a]
Test weight (lbs/bu)	60.7	0.8	60.1	61.7	0.4	0.8	59.5	61.9	0.8
Wheat protein (%)	14.8	1.4	13.2	15.8	0.3	0.6	13.6	15.7	0.7
Flour yield (%)	57.1	1.3	56.2	58.6	NS	2.0	53.5	60.7	NS
Mixograph pattern	4.8	0.3	4.5	5.2	NS	0.9	3.3	7.0	1.0
Bread-making									
Water absorption (%)	63.8	0.7	63.1	64.4	NS	1.4	62.0	67.3	2.0
Dough handling	9.8	0.2	9.6	10.0	NS	0.2	9.7	10.0	NS
Loaf volume (cm^3)	188.1	11.1	175.3	194.5	6.3	5.9	180.7	197.0	NS
Crumb color	7.8	0.4	7.4	8.0	0.4	0.4	7.3	8.3	NS
Crust color	9.8	0.2	9.6	10.0	NS	0.3	9.0	10.0	NS
FAC (μg/g)	617.1	1.2	503.1	781.1	–	1.3	346.9	886.2	–
Natural logarithm (FAC)	6.4	0.2	6.2	6.7	0.3	0.3	5.8	6.8	0.2

[a] LSD = least significant difference at P = 0.05, NS = not significant (P > 0.05).

Table 2
Least square means of concentration of SDS extractable and unextractable protein fractions analyzed by size exclusion HPLC based on whole wheat (% Wheat) and HPLC absorbance area (% Area) for hard red spring wheat genotypes grown at three locations in North Dakota.[a]

	(% Wheat)						(% Area)					
	Extractable			Unextractable			Extractable			Unextractable		
Sample	EF1	EF2	EF3	UF1	UF2	UF3	EP1	EP2	EP3	UP1	UP2	UP3
Location												
Carrington	1.97	6.19	2.51	2.61	1.69	0.77	12.5	39.3	16.0	16.6	10.7	4.9
Casselton	1.66	4.99	2.22	2.17	1.47	0.70	12.6	37.8	16.9	16.4	11.1	5.3
Langdon	2.02	6.11	2.55	2.45	1.67	0.78	13.0	39.2	16.4	15.7	10.7	5.0
LSD[b]	0.17	0.39	0.15	0.16	NS	NS	NS	NS	NS	NS	NS	NS
Genotype												
Glenn	1.92	6.07	2.51	2.79	1.60	0.77	12.2	38.7	16.0	17.8	10.3	5.0
Faller	1.76	5.07	2.38	2.19	1.45	0.71	13.0	37.4	17.6	16.2	10.6	5.2
V01	1.75	5.86	2.47	2.26	1.69	0.78	11.8	39.6	16.7	15.3	11.4	5.2
V02	1.84	5.92	2.44	2.36	1.72	0.79	12.2	39.2	16.2	15.6	11.4	5.3
V03	2.04	6.00	2.43	2.30	1.43	0.70	13.7	40.2	16.3	15.4	9.6	4.7
V04	1.91	5.80	2.47	2.67	1.64	0.75	12.5	38.0	16.2	17.5	10.8	4.9
V05	1.94	6.02	2.37	2.53	1.74	0.76	12.6	39.1	15.4	16.5	11.4	5.0
V06	1.85	5.91	2.45	2.20	1.83	0.79	12.3	39.2	16.3	14.6	12.2	5.3
V07	1.86	5.44	2.38	2.23	1.47	0.72	13.2	38.4	16.9	15.8	10.5	5.1
V08	1.95	5.82	2.38	2.53	1.64	0.74	12.9	38.6	15.9	16.8	10.9	4.9
V09	1.90	5.49	2.43	2.45	1.51	0.72	13.1	37.8	16.8	16.9	10.4	5.0
LSD[b]	0.11	0.31	NS	0.12	NS	NS	0.7	NS	0.7	0.9	NS	NS

[a] Please refer Fig. 1 for EF1-3 and UF1-3.; and EP1-3 and UP1-3 = HPLC absorbance area percent values based on total area for EF1-3 and UF1-3, respectively.
[b] LSD = least significant difference at P = 0.05, NS = not significant (P > 0.05).

Table 3
Mean square values for percent of SDS extractable and unextractable protein fractions based on whole wheat (% Wheat) and HPLC absorbance area (% Area).[a]

Source of Variance	Location (L)	Error I [Rep. (L)]	Genotype (G)	G × L Interaction	Error II (Residual)
Parameters\ DF[b]	2	6	10	20	60
% Wheat					
Extractable					
EF1	1.23**	0.08	0.06***	0.01^{ns}	0.01
EF2	14.95***	0.41	0.84***	0.12^{ns}	0.11
EF3	1.04**	0.06	0.02^{ns}	0.02*	0.01
Unextractable					
UF1	1.69**	0.07	0.36***	0.02^{ns}	0.02
UF2	0.49^{ns}	0.40	0.16^{ns}	0.10^{ns}	0.11
UF3	0.07^{ns}	0.05	0.01^{ns}	0.01^{ns}	0.01
% Area					
Extractable					
EP1	1.67^{ns}	4.00	2.70***	0.41^{ns}	0.60
EP2	25.05^{ns}	19.01	6.06^{ns}	3.58^{ns}	4.68
EP3	6.54^{ns}	3.33	2.93***	0.49^{ns}	0.58
Unextractable					
UP1	6.59^{ns}	3.85	8.61***	0.47^{ns}	0.81
UP2	1.74^{ns}	18.68	4.46^{ns}	3.55^{ns}	4.70
UP3	1.36^{ns}	2.51	0.28^{ns}	0.27^{ns}	0.34

*, **, and ***: F-value is significant at P < 0.05, P < 0.01 and P < 0.001, respectively. ns = not significant (P > 0.05).
[a] Please refer Fig. 1 for EF1-3 and UF1-3.; and EP1-3 and UP1-3 = HPLC absorbance area percent values based on total area for EF1-3 and UF1-3, respectively.
[b] DF = degree of freedom.

consistent in rankings for those protein MWD parameters across three locations in this research.

3.3. Correlations between protein MWD parameters and quality characteristics

Phenotypic and genotypic correlations were estimated to evaluate the associations between protein MWD parameters and quality characteristics. The polymeric protein parameters were identified to have significant correlations with quality traits in other researches (Gupta et al., 1993; Ohm et al., 2009; Park et al., 2006). However, those results were mainly based on simple linear correlations. In the current research, we estimated phenotypic and genotypic correlations of polymeric protein parameters with quality characteristics to examine the usefulness of those parameters as indirect indexes to screen experimental lines for quality improvement in HRS wheat breeding (Table 4).

Wheat protein concentration showed significant (P < 0.05) r_p and r_g values with flour yield, mixograph pattern, and bread loaf volume (Table 4). These results indicate that quantitative increase of total protein in wheat resulted in decreased flour yield however; and it also improved mixing property and bread loaf volume for wheat genotypes in this sample set. The polymeric proteins of EXP appeared to have negative influence on mixing and bread-making quality despite the positive r_p value between wheat protein concentration and EF1. EP1 had especially significant and negative r_p values with wheat protein, mixograph pattern, and bread loaf volume, which would suggest that a high proportion of extractable polymeric proteins might have an adverse influence on mixing and bread-making quality. However, EF1 and EP1 did not have

Table 4
Phenotypic and genotypic correlations of wheat protein content and polymeric protein parameters with quality traits and free asparagine concentration.[a]

Variables	Wheat Protein (%)	EF1 (% Wheat)	EP1 (% Area)	UF1 (% Wheat)	UP1 (% Area)
	Phenotypic correlation coefficient				
Test weight	0.06^{ns}	-0.17^{ns}	-0.23^{ns}	0.16^{ns}	0.15^{ns}
Flour yield	-0.46^{*}	-0.02^{ns}	0.42^{*}	-0.35^{ns}	-0.14^{ns}
Wheat protein	–	0.49^{*}	-0.41^{*}	0.69^{**}	0.23^{ns}
Mixograph pattern	0.78^{**}	0.19^{ns}	-0.51^{*}	0.78^{**}	0.50^{*}
Water absorption	0.05^{ns}	-0.36^{ns}	-0.44^{*}	-0.12^{ns}	-0.21^{ns}
Bread loaf volume	0.50^{*}	0.00^{ns}	-0.45^{*}	0.54^{**}	0.38^{ns}
Free asparagine	-0.03^{ns}	-0.23^{ns}	-0.22^{ns}	-0.59^{**}	-0.77^{**}
	Genotypic correlation coefficient				
Test weight	0.27^{ns}	-0.14^{ns}	-0.41^{ns}	0.27^{ns}	0.20^{ns}
Flour yield	-0.83^{*}	-0.12^{ns}	0.66^{ns}	-0.49^{ns}	-0.13^{ns}
Wheat protein	–	0.49^{ns}	-0.44^{ns}	0.72^{*}	0.34^{ns}
Mixograph pattern	0.91^{**}	0.34^{ns}	-0.51^{ns}	0.87^{**}	0.59^{ns}
Water absorption	0.05^{ns}	-0.79^{*}	-0.86^{**}	-0.18^{ns}	-0.28^{ns}
Bread loaf volume	0.65^{*}	0.01^{ns}	-0.61^{ns}	0.86^{**}	0.75^{*}
Free asparagine	-0.37^{ns}	-0.57^{ns}	-0.26^{ns}	-0.85^{*}	-0.92^{**}

* and **: Correlation coefficient is significant at $P < 0.05$ and $P < 0.01$, respectively. ns = not significant ($P > 0.05$).
[a] Please refer Fig. 1 for EF1 and UF1.; and EP1 and UP1=HPLC absorbance area percent values based on total area for EF1 and UF1, respectively.

significant ($P > 0.05$) r_g values with the quality characteristics with the exception of water absorption. Phenotypic correlation is influenced by factors such as GE interaction as well as genotypic variation. Therefore, EF1 and EP1 may not be functional enough to be used in screening lines for quality characteristics with the exception of water absorption.

Among SDS unextractable polymeric protein parameters, UF1was significantly and positively correlated with wheat protein, mixograph pattern, and bread loaf volume (Table 4). Specifically, the r_g values were significant ($P < 0.05$) between UF1 and those quality characteristics (Table 4). The significant r_g value that exists between UF1 and wheat protein concentration indicates that the positive effect of wheat protein concentration on mixing and bread loaf volume was substantially associated with quantitative variation of polymeric proteins of UNP for genotypes in this research.

3.4. Correlation between free asparagine concentration and protein MWD parameters

Simple linear correlations were estimated between wheat FAC and A% values of EXP and UNP fractions analyzed using an SE-HPLC as displayed in Fig. 1. While EXP fractions showed low correlations, the SDS unextractable polymeric protein fractions were identified to have recognizable negative correlations with FAC in this experiment. The A% value of SDS unextractable polymeric protein fractions showed particularly significant ($P < 0.001$) and negative correlations with FAC in this research (Fig. 1). Thus, the lower FAC was associated with the higher proportion of SDS unextractable polymeric proteins in total proteins. Simsek et al. (2014) also reported a similar result for HRS wheat sample set that was damaged by sprouting.

The FAC also showed significant ($P < 0.05$) r_p and r_g values with UF1 and UP1 (Table 4). FAC especially had negative genotypic correlations with UF1 ($r_g = -0.85$, $P < 0.05$) and UP1 ($r_g = -0.92$, $P < 0.01$) (Table 4). Therefore, the correlation between SDS unextractable polymeric proteins and FAC can be explained by common genetic factors that have strong influences on those two traits. One of the possible common factors might be variation of endo-protease activity in HRS wheat genotypes. The endo-protease activity was observed to have positive correlations with degradation of polymeric proteins and FAC for HRS wheat genotypes by Simsek et al. (2014). However, this result was obtained from a HRS wheat sample set that was subjected to artificial sprouting. Therefore, further research should be necessary to confirm the causative influence of endo-protease activity on the association between polymeric proteins and FAC for non-sprouted HRS wheat samples.

4. Conclusions

Few reports are available for the effect of the genotypes on the

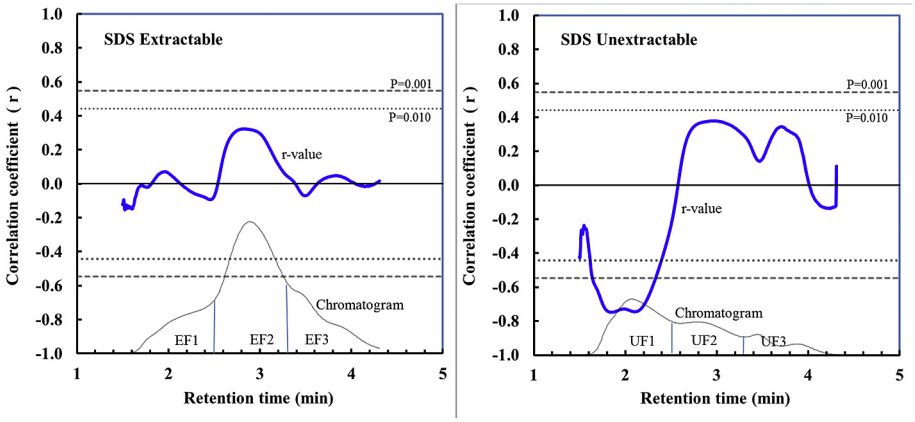

Fig. 1. Spectrum of linear correlation coefficients between free asparagine concentration and UV absorbance area % values of size exclusion HPLC for SDS extractable and unextractable protein fractions.

associations of protein MWD parameters with quality characteristics and FAC in HRS wheat grown in ND. Among protein MWD parameters, a quantitative parameter of polymeric proteins in the SDS unextractable fraction (UF1) was identified to have significant and positive genotypic correlations with mixing and bread-making quality characteristics including mixograph pattern and bread loaf volume. The FAC also showed significant and negative genotypic correlations with SDS unextractable polymeric protein parameters (UF1 and UP1). The results supported the idea that the SDS unextractable polymeric proteins were highly associated with mixing and bread-making quality characteristics and FAC due to genotypic variations. The ANOVA showed that there was significant ($P < 0.01$) variation in growing locations and genotypes for SDS unextractable polymeric protein parameters while there was a non-significant ($P > 0.05$) influence of genotype by location interaction. Overall, these results indicate that SDS unextractable polymeric protein parameters (UF1 and UP1) are useful to screen wheat genotypes indirectly for low FAC in addition to excellent bread-making quality in HRS wheat breeding. The quality evaluation and FAC analysis of wheat samples are very laborious and time-consuming. The analysis of FAC requires further complex analytical instruments. The protein MWD analysis was performed by an SE-HPLC using a microbore column, which requires a small quantity of sample (10 mg) and a short analysis time (5 min) in the current research. Therefore, it is most likely to be helpful to screen a large number of genotypes for high bread-making quality and low FAC if sample quantity is limited in HRS wheat breeding.

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